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16. ABSTRACT

Dietary concentrations of 0, 20, 100, and 500 ppm of technical grade pentachlorophenol were fed to male and female Sherman strain rats for 8 months. The same experiment using purified pentachlorophenol was carried out. The food consumption was measured in all rats during the second week of exposure and for one week every 6 weeks thereafter. An autopsy was performed on all rats at the end of the experiment. The brain, lungs, spleen, liver, kidneys, heart, and testes were weighed and examined grossly and microscopically in all rats fed purified pentachlorophenol, all female rats fed technical pentachlorophenol, and in the male rats fed the highest dose of technical pentachlorophenol and the controls. Only the kidneys and livers were examined microscopically in the male rats fed 20 and 100 ppm of technical pentachlorophenol. Although the food intake was comparable, male and female rats fed 500 ppm of technical and male rats fed 500 ppm of purified pentachlorophenol gained less weight. The livers of the male and female rats red 500 ppm technical pentachlorophenol weighed significantly more than those of the controls. The kidneys of all male rats fed purified pentachlorophenol weighed significantly more than those of the controls; however, there was no dose-related increase. No morphological changes were seen in the kidneys. At the 500-ppm dietary concentrations, technical pentachlorophenol produced a severe effect in the liver of female rats characterized by vacuolation of the hepatocytes, an increase in fibroblasts and other mononuclear cells within sinusoids, bile duct proliferation, periportal fibrosis, degenerated liver cells, increased mitotic figures, and an accumulation of brown pigment in macrophages and in Kupffer cells. In male rats at the 100- or 500-ppm dietary concentrations of technical pentachlorophenol, the predominant lesion consisted of enlarged pleomorphic hepatocytes which had foamy cytoplasm or cytoplasm with large vacuoles. The walls of the hepatic central veins of the livers in animals of both sexes were thickened. At the 100-ppm dietary concentrations similar but less pronounced effects were observed in the livers. Only mild alterations were noted at the 20-ppm dietary concentration. Purified pentachlorophenol caused slightly enlarged liver cells with occasional eosinophilic cytoplasmic inclusions at 500 ppm but no alterations were observed in the livers of rats fed the 100- and 20-ppm dietary concentrations. The results suggest that most of the toxicity associated with feeding technical grade pentachlorophenol to rats at these dietary concentrations stems from toxic contaminants rather than from pentachlorophenol.

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The Effect of Technical and Purified Pentachlorophenol on the Rat Liver[1]

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The Effect of Technical and Purified Pentachlorophenol on the Rat Liver. KIMBROUGH, R. D., AND LINDER, R. E. (1978). *Toxicol. Appl. Pharmacol.* **46**, 151–162. Dietary concentrations of 0, 20, 100, and 500 ppm of technical grade pentachlorophenol were fed to male and female Sherman strain rats for 8 months. The same experiment using purified pentachlorophenol was carried out. The food consumption was measured in all rats during the second week of exposure and for one week every 6 weeks thereafter. An autopsy was performed on all rats at the end of the experiment. The brain, lungs, spleen, liver, kidneys, heart, and testes were weighed and examined grossly and microscopically in all rats fed purified pentachlorophenol, all female rats fed technical pentachlorophenol, and in the male rats fed the highest dose of technical pentachlorophenol and the controls. Only the kidneys and livers were examined microscopically in the male rats fed 20 and 100 ppm of technical pentachlorophenol. Although the food intake was comparable, male and female rats fed 500 ppm of technical and male rats fed 500 ppm of purified pentachlorophenol gained less weight. The livers of the male and female rats fed 500 ppm technical pentachlorophenol weighed significantly more than those of the controls. The kidneys of all male rats fed purified pentachlorophenol weighed significantly more than those of the controls; however, there was no dose-related increase. No morphological changes were seen in the kidneys. At the 500-ppm dietary concentrations, technical pentachlorophenol produced a severe effect in the liver of female rats characterized by vacuolation of the hepatocytes, an increase in fibroblasts and other mononuclear cells within sinusoids, bile duct proliferation, periportal fibrosis, degenerated liver cells, increased mitotic figures, and an accumulation of brown pigment in macrophages and in Kupffer cells. In male rats at the 100- or 500-ppm dietary concentrations of technical pentachlorophenol, the predominant lesion consisted of enlarged pleomorphic hepatocytes which had foamy cytoplasm or cytoplasm with large vacuoles. The walls of the hepatic central veins of the livers in animals of both sexes were thickened. At the 100-ppm dietary concentrations similar but less pronounced effects were observed in the livers. Only mild alterations were noted at the 20-ppm dietary concentration. Purified pentachlorophenol caused slightly enlarged liver cells with occasional eosinophilic cytoplasmic inclusions at 500 ppm but no alterations were observed in the livers of rats fed the 100- and 20-ppm dietary concentrations. The results suggest that most of the toxicity associated with feeding technical grade pentachlorophenol to rats at these dietary concentrations stems from toxic contaminants rather than from pentachlorophenol.

Pentachlorophenol is an antimicrobial registered by the U.S. Environmental Protection Agency (EPA) for use in agricultural seed treatment, on cordage and leather, masonry,

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in paints and varnishes, on cable covering, nets, for mold control, in drilling and production of petroleum, and as a wood preservative. Technical grades of pentachlorophenol may contain other chlorinated phenols as well as neutral impurities, such as chlorinated dibenzodioxins, chlorinated dibenzofurans, and chlorinated diphenyl ethers (Goldstein *et al.*, 1977; Johnson *et al.*, 1973). Johnson *et al.* (1973) compared the toxic effects of a technical pentachlorophenol which contained 1980 ppm of octachlorodibenzodioxin and 19 ppm of hexachlorodibenzodioxin with those of a pure pentachlorophenol. They noted a positive response for the chick edema assay and the rabbit ear bioassay with the technical pentachlorophenol but not with the pure pentachlorophenol. In a rat feeding study at dosage levels of 30, 10, and 3 mg/kg body weight/day the technical as well as the pure pentachlorophenol produced an increase in the liver and kidney weights at the higher dosage levels. The technical pentachlorophenol resulted in focal hepatocellular degeneration and necrosis, while no microscopic alterations were noted in rats that were fed pure pentachlorophenol.

To elucidate this problem further, we fed rats diets containing purified or technical pentachlorophenol. In a preliminary study (Kimbrough and Linder, 1975), 1000 ppm of purified or technical pentachlorophenol was fed in the diet to rats for 3 months and marked alterations were noted in the livers of rats fed the technical pentachlorophenol. For this reason lower dietary concentrations of purified and technical pentachlorophenol were fed to rats for 8 months. It is the purpose of this paper to report the results.

METHODS

Weaning, randomly bred, cesarean-derived and barrier-sustained Sherman strain rats from the colony at the Center for Disease Control, U.S. DHEW, were randomly distributed into groups of 10 rats of each sex. The rats have been randomly bred since 1950. They are descendants of the Sherman strain developed at Columbia University from Osborne–Mendel rats (Kimbrough *et al.*, 1964). The rats were fed diets *ad libitum* that contained concentrations of 0, 20, 100, and 500 ppm of technical or purified pentachlorophenol for 8 months. A separate control group was used for the study of technical pentachlorophenol and for the study of purified pentachlorophenol. Diets were prepared by pulverizing and thoroughly mixing the pentachlorophenol into 10 g of corn starch with a mortar and pestle. The pentachlorophenol/starch mixture was then blended into enough ground laboratory chow to give 450 g of concentrated diet. Final concentrations were prepared in a planetary-type electric mixer by making successive dilutions with ground chow. The mixing procedure was repeated about every 2 weeks. Final diet preparations were kept in covered metal containers. Feed cups were replenished daily and changed weekly. Since the technical pentachlorophenol was prilled, it was pulverized first. The technical pentachlorophenol was from Monsanto Chemical Co., Lot No. KA578, and the purified pentachlorophenol was from Aldrich Chemical Co. Preliminary results in our laboratory showed the presence of chlorinated dibenzofurans and chlorinated dibenzodioxins in the technical pentachlorophenol. The technical and purified preparations of pentachlorophenol were also analyzed for impurities at the National Institute of Environmental Health Sciences. The method employed for this analysis and the results obtained have been published (Goldstein *et al.*, 1977). Briefly, the technical pentachlorophenol contained 84.6% pentachlorophenol and 3% of 2, 3, 4,

6% tetrachlorophenol. Other impurities detected but not quantitated include chlorodiphenyl ethers and aliphatic nonhalogenated compounds. The technical pentachlorophenol also contained 8 ppm of hexachlorodibenzodioxin, 520 ppm of heptachlorodibenzodioxin, 1380 ppm of octachlorodibenzodioxin, 260 ppm of octachlorodibenzofuran, 400 ppm of heptachlorodibenzofuran, 90 ppm of hexachlorodibenzofuran, 40 ppm of pentachlorodibenzofuran, and 4 ppm of tetrachlorodibenzofuran. The purified pentachlorophenol (purity greater than 99%) contained less than 0.1 ppm of the combined halogenated dibenzodioxins and dibenzofurans (Goldstein *et al.*, 1977).

The rats were kept in group cages; they were weighed weekly until 4 months old and then biweekly. The food consumption was measured in all rats during the second week of exposure and for a week every 6 weeks thereafter. Care was taken to measure and estimate any spillage. All rats were sacrificed 8 months after onset of exposure. They were anesthetized with ether, a midline incision was made into the abdominal wall, the aorta was cut, and the rats were exsanguinated. Exsanguination avoids congestion of organs, which interferes with organ weights and microscopic examination of the organs. An autopsy was performed; the brain, lungs, heart, spleen, liver, kidneys, and testes were weighed and fixed in buffered 4% formaldehyde for microscopical examination. Thyroid, parathyroid, gastrointestinal tract, all reproductive organs, bladder, and the adrenals of the controls and the rats fed the purified pentachlorophenol were also examined microscopically. Brain, lungs, heart, spleen, liver, and kidneys were examined of all male and female rats fed the technical pentachlorophenol with the exception of the male rats fed the 20- and 100-ppm dietary concentrations. In these two groups only the liver and the kidneys were examined microscopically after it was established that no effect in the other organs was noted in the females or the males fed either 500 ppm of technical pentachlorophenol in this experiment or 1000 ppm in an earlier experiment (Kimbrough and Linder, 1975). All tissues were stained with hematoxylin and eosin, and occasionally livers were stained with periodic acid–Schiff stain (P.A.S.) without diastase and a Perl stain for iron. Body weight and organ weights were compared by analysis of variance, followed by the Duncan multiple range test (Steele and Torrie, 1960).

RESULTS

None of the rats died during the study and no signs of toxicity were noted.

The approximate daily dosages of technical and purified pentachlorophenol consumed by the rats are given in Fig. 1. With increasing age of the rats, the food consumption decreased but no appreciable difference was noted between rats fed the diets containing different concentrations of pentachlorophenol or between rats given the technical grade or purified pentachlorophenol. Since rats in the groups fed 500 ppm of pentachlorophenol (technical and purified) had gained less weight their food consumption relative to body weight was consistently greater (Figs. 2 and 3).

Final body weights, liver weights, and kidney weights are listed in Table 1. All rats in groups fed 500 ppm of either grade of pentachlorophenol gained less weight than rats in the respective control groups; the effect on females fed the purified material was slight. A significant increase in liver weight occurred in male and female rats fed 500 ppm of technical pentachlorophenol. Kidneys of male rats fed all three dietary levels of purified pentachlorophenol weighed significantly more than kidneys of male rats in the

appropriate control group; however, the increase in weight was not dose related. Dose-related effects on weights of testes, lungs, heart, spleen, and brain were not observed. No attempt was made to compare relative organ weights because of the unequal weight gain in the groups fed 500 ppm.

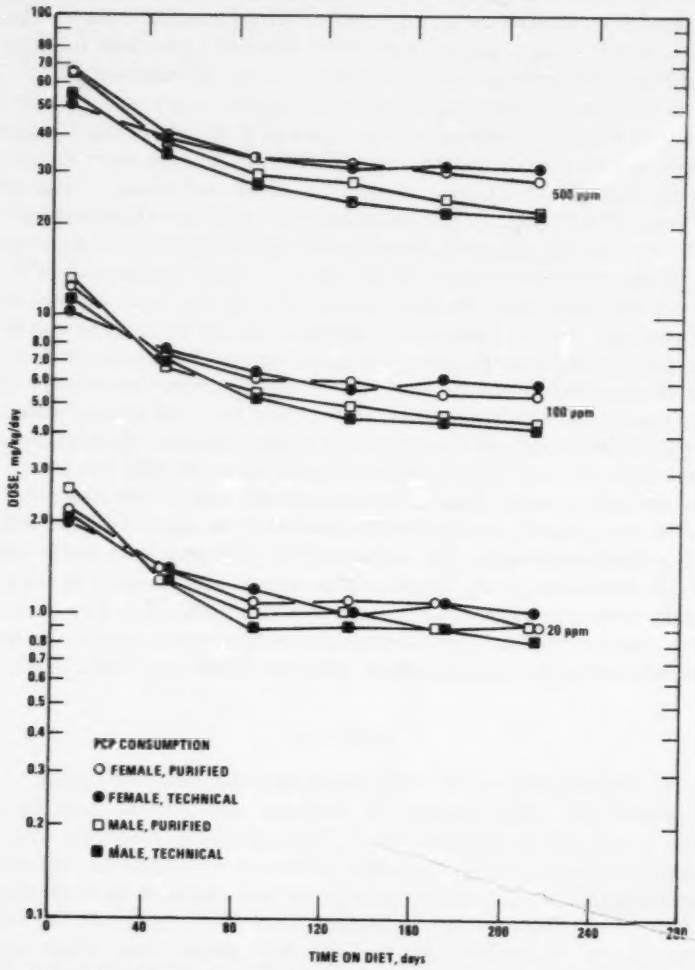

FIG. 1. Consumption of pentachlorophenol (PCP) on a milligram per kilogram body weight basis.

On gross inspection, a slightly brownish diffuse discoloration was observed in the livers of six female rats fed 500 ppm of purified pentachlorophenol. These livers did not fluoresce when they were exposed to uv light (366 nm), and the livers from the males or females fed the lower dietary concentrations of purified pentachlorophenol were normal grossly. The livers of eight female rats fed 100 ppm of technical pentachlorophenol and

of six female rats fed 500 ppm of technical pentachlorophenol were black. All black livers fluoresced pink under uv light, indicative of hepatic porphyria (Goldstein *et al.*, 1977).

The liver was the only organ that showed alterations on microscopic examination which were related to ingestion of pentachlorophenol. The livers of all control rats and all rats fed 20 or 100 ppm of purified pentachlorophenol were normal. Hepatocytes

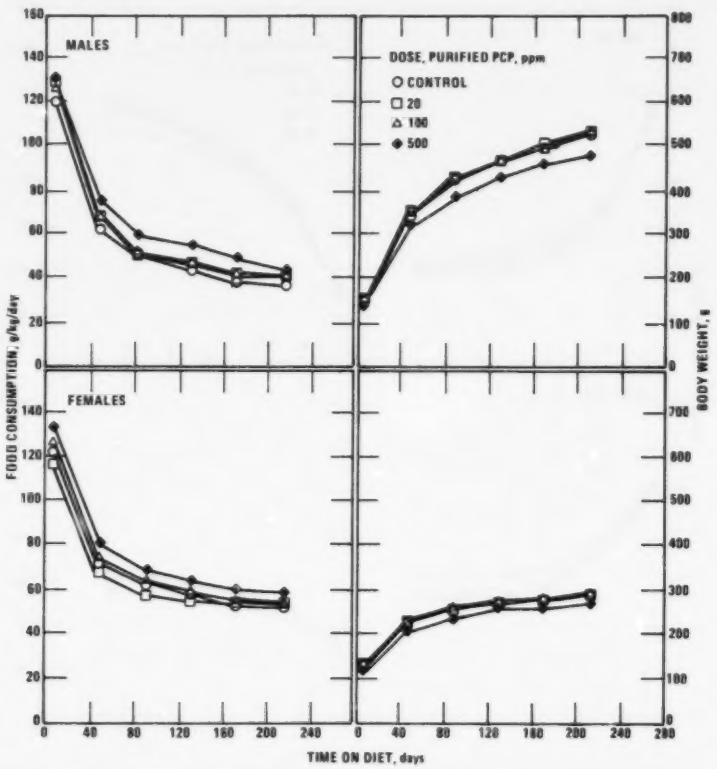

FIG. 2. Food consumption and body weight of rats fed purified pentachlorophenol (PCP).

around central veins were slightly enlarged in the livers of eight male rats and five female rats fed 500 ppm of purified pentachlorophenol. At this dosage level cytoplasmic eosinophilic inclusions were occasionally noted in the liver cells of five male rats and a brown pigment was present in macrophages of the livers of three female rats.

In contrast the rats fed the technical grade pentachlorophenol (Fig. 4–7) had prominent hepatic lesions at the higher dosage levels. Centrolobular hepatocytes were slightly enlarged and occasionally vacuolated in all male rats and in one female fed 20 ppm. At the 100-ppm dietary level the hepatocytes were enlarged in all male and female rats and showed marked coarse and fine vacuolation of the cytoplasm in 7 females and

10 males. Brown pigment was abundant in macrophages and in Kupffer cells of nine females. In liver sections of seven females, some hepatocytes had pyknotic nuclei and highly eosinophilic-staining smooth cytoplasm. Some sinusoids contained hyaline bodies or fragments of hepatocellular cytoplasm with nuclei displaying karyorrhexis. Mitotic figures were noted in three females. Periportal fibrosis was observed in portal triads and generally in the peripheral areas of the liver lobules in four female rats. The

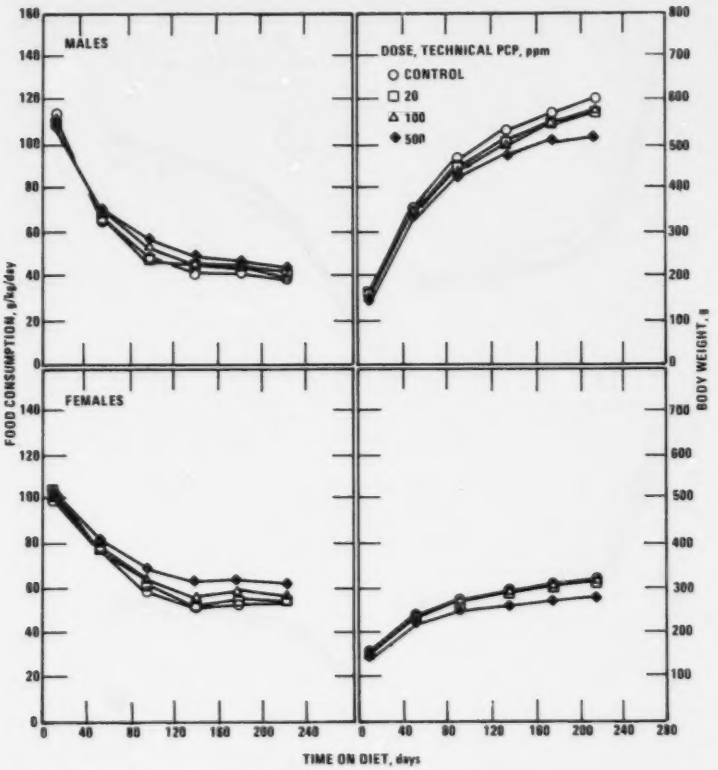

FIG. 3. Food consumption and body weight of rats fed technical pentachlorophenol (PCP).

central veins were generally slightly thickened by an increase in fibrous tissue. A small neoplastic nodule was present in one liver. Neoplastic nodules (hyperplastic nodules) have been defined previously (Kimbrough *et al.*, 1975).

At the 500-ppm dietary concentration of technical pentachlorophenol the morphological changes were even more pronounced. The thickening of the wall of the central veins by fibrosis was more extensive. The hepatocytes were hypertrophic and finely or coarsely vacuolated (Fig. 4) in all male and female rats. Periportal fibrosis was present in the livers of nine females and four males (Fig. 5), and was accompanied by bile duct proliferation in six female rats. Pleomorphic hepatocytes with hyperchromatic nuclei or

multinucleation (Fig. 7) was observed in the livers of almost all female rats and half of the males. In all 10 female and in five male rats (Fig. 6) an abundant amount of brown pigment was present in macrophages and Kupffer cells. The pigment stained positive with P.A.S. and Perl's stain, indicating, that this material at least in part represents ceroid pigment, which is composed of lipid pigments associated with hemosiderin (Hartcroft, 1972). In experimental hepatic porphyria porphyrins accompany the ceroid pigment (Kimbrough *et al.*, 1973). In females fed 500 ppm of technical pentachlorophenol hepatocytes with karyorrhectic or pyknotic nuclei and dense cytoplasm as well

TABLE 1

BODY, LIVER, AND KIDNEY WEIGHTS OF RATS FED TECHNICAL OR PURIFIED PENTACHLOROPHENOL FOR 8 MONTHS

Compound	Concentration in the diet (ppm)	Sex	Average final body weight (g)	Average liver weight (g)	Average kidney weight (g)
Technical pentachlorophenol	0	M	626 ± 26[a]	16.08 ± 0.76	3.07 ± 0.14
	20	M	604 ± 14	16.31 ± 0.66	2.89 ± 0.09
	100	M	587 ± 17	15.57 ± 0.67	2.96 ± 0.09
	500	M	534 ± 22[b]	21.64 ± 1.27[b]	3.30 ± 0.17
	0	F	322 ± 4	8.78 ± 0.28	2.09 ± 0.06
	20	F	327 ± 7	9.14 ± 0.21	1.94 ± 0.04
	100	F	328 ± 13	10.15 ± 0.53	1.90 ± 0.06
	500	F	269 ± 9[b]	12.22 ± 0.75[b]	1.87 ± 0.05
Purified pentachlorophenol	0	M	550 ± 15	12.31 ± 0.39	2.58 ± 0.10
	20	M	555 ± 20	14.23 ± 0.55	3.03 ± 0.10[b]
	100	M	558 ± 15	13.83 ± 0.63	3.01 ± 0.12[b]
	500	M	495 ± 17[b]	13.41 ± 0.54	3.00 ± 0.08[b]
	0	F	286 ± 8	7.12 ± 0.22	1.71 ± 0.05
	20	F	292 ± 11	7.35 ± 0.39	1.71 ± 0.05
	100	F	290 ± 8	7.69 ± 0.25	1.80 ± 0.04
	500	F	271 ± 7	8.03 ± 0.28	1.87 ± 0.05

[a] Values are group means ± SE.
[b] Different from control group, $p < 0.05$.

as hyaline bodies were observed, and a number of mitotic figures were also seen. The outer surface of the liver in female rats was irregular with pitted areas of retraction. Focal areas of localized fibrosis were noted. The hepatocytes generally showed a great deal of pleomorphism and the cytoplasm varied in its affinity for eosin. The hepatocytes surrounding the central veins stained more basophilic. In addition, small areas of adenofibrosis were observed in two female rats. Adenofibrosis represents a proliferation of epithelial cells that form ducts and that are surrounded by fibrosis (Kimbrough *et al.*, 1973).

Except for occasional control and treated rats with chronic nephrosclerosis and occasional rats with pulmonary fibrosis unrelated to treatment no other abnormal

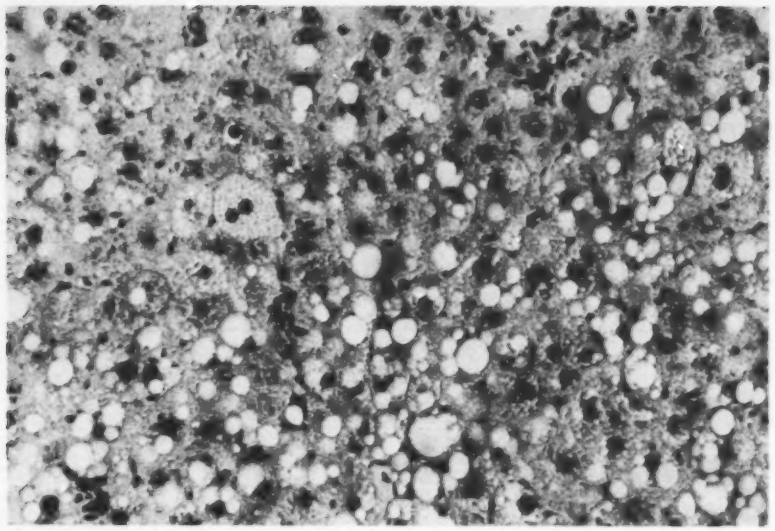

FIG. 4. Photomicrograph of liver from a male rat fed 500 ppm of technical grade pentachlorophenol. Many hepatocytes contain large and small vacuoles or have a foamy cytoplasm (H + E, × 100).

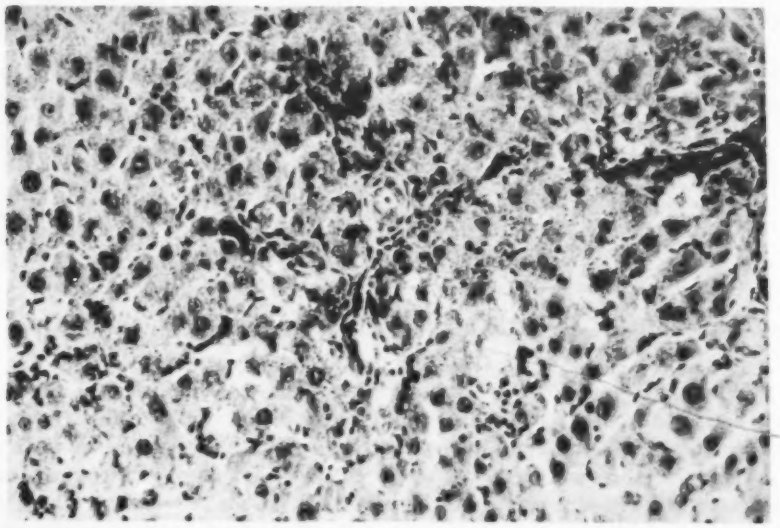

FIG. 5. Photomicrograph of liver from a female rat fed 500 ppm of technical pentachlorophenol. Note the cellular pleomorphism, interstitial fibrosis, accumulation of inflammatory cells, and the small homogeneously appearing areas which represent pigment (H + E, ×100).

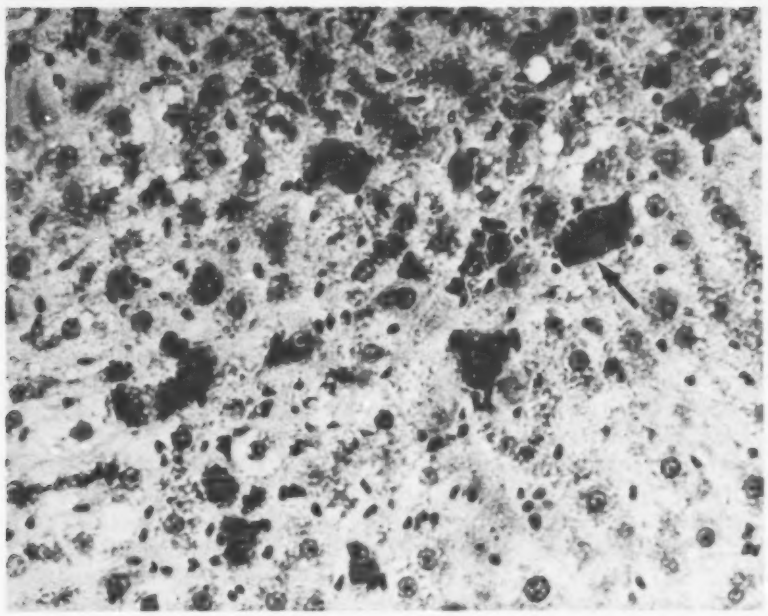

FIG. 6. Photomicrograph of liver from a female rat fed 500 ppm of technical pentachlorophenol illustrating black pigment in more detail (H + E, ×100).

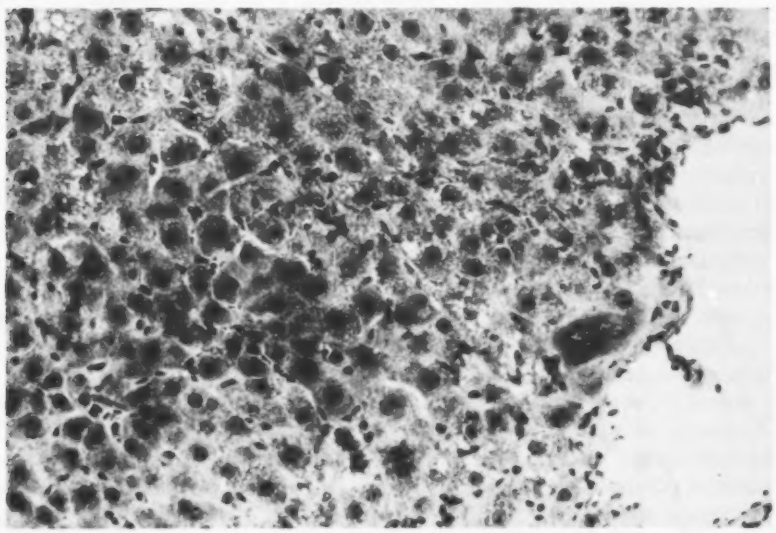

FIG. 7. Photomicrograph of liver showing cellular pleomorphism hepatocytes with hyperchromatic nuclei and darker staining cytoplasm, and a multinucleated cell (H + E, ×100).

lesions were found. The increase in the weight of the kidneys could not be correlated with any abnormal microscopic findings.

DISCUSSION

Johnson *et al.* (1973) reported that feeding pure pentachlorophenol to rats for 90 days resulted in increased kidney and liver weights at dietary levels equivalent to 30 mg/kg/day and increased liver weight at 10 mg/kg/day, but observed no gross or microscopic lesions. In our 8-month study the livers from rats fed 500 ppm of purified pentachlorophenol also showed only minor hepatocellular alterations.

The light microscope findings made in the liver of rats treated with purified pentachlorophenol are consistent with an increase in smooth endoplasmic reticulum and the formation of myelin figures. Ultrastructural studies conducted earlier on liver of rats fed 1000 ppm of purified pentachlorophenol (Kimbrough and Linder, 1975) illustrate this. An increase in smooth endoplasmic reticulum and the myelin figures may be reversible after cessation of treatment.

The effects of technical pentachlorophenol on the liver were pronounced at the dietary levels of 100 and 500 ppm and minor changes were still present at 20 ppm. At 100 ppm the adult rats consumed roughly 5 mg of pentachlorophenol/kg body weight/day. Five milligrams of technical pentachlorophenol contains a combined concentration of about 13 μg of chlorinated dibenzodioxins and chlorinated dibenzofurans. Thus, these highly toxic trace contaminants may have been responsible for most of the alterations noted in the liver of the exposed rats. The pathological changes in the liver produced by technical pentachlorophenol probably represent a permanent lesion. Particularly, the irregular outer surface of the liver indicates that proliferation, atrophy, and regression had occurred in the livers of treated rats. The adenofibrosis (a proliferation of epithelial cells forming ducts which are surrounded by fibrosis) has been produced by a number of other chemicals in rats (Kimbrough *et al.*, 1973). Adenofibrosis in rodents usually occurs concomitantly with hepacellular carcinoma and is also a persistent lesion. In rats fed polychlorinated biphenyls for 6 months adenofibrosis persisted for a 10-month recovery period (Kimbrough *et al.*, 1973). However, the chemicals fed to the rats could still be detected in the livers at the end of the recovery period, which may have an influence on the lesion. Thickening of the vascular wall of the central veins by fibrosis also represents a permanent lesion which has previously been reported in rats exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Jones and Butler, 1974).

A number of aromatic compounds produce hepatic porphyria in female rats, while male rats do not develop hepatic porphyria. This sex difference in rats is presently not well understood and not all species studied show it (Strik, 1973). Some species are more susceptible to the induction of hepatic porphyria than others. Jones and Sweeney (1977) recently showed that mice genetically responsive to the induction of arylhydrocarbon hydroxylase when treated with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin developed experimental porphyria while mice that were unresponsive to the induction of aryl hydrocarbon hydroxylase did not develop experimental porphyria when 2,3,7,8-tetrachlorodibenzo-*p*-dioxin was administered to them. The nonresponsive mice had normal hepatic uroporphyrinogen decarboxylase activity while it was decreased in the

responsive mice. Goldstein *et al.* (1977) determined in female rats, fed the same technical pentachlorophenol used in this study, that the total liver prophyrins were elevated and that pink fluorescence of the liver under uv light as reported in this study was consistent with the presence of porphyrins. None of the livers from rats fed the purified pentachlorophenol showed elevated liver porphyrins. While technical pentachlorophenol increased hepatic aryl hydrocarbon hydroxylase activity significantly, this was not true for purified pentachlorophenol. These findings are consistent with those of Jones and Sweeney (1977), namely, that induction of aryl hydrocarbon hydroxylase may be one prerequisite for the development of experimental porphyria. The morphological alterations produced by technical pentachlorophenol and the effect on certain liver enzymes (aryl hydrocarbon hydroxylase, cytochrome *P*-450, glucuronyl transferase, porphyrin metabolism) reported by Goldstein *et al.* (1977) demonstrate that the more severe morphological alterations observed microscopically are accompanied by a derangement of the liver enzyme system.

Pentachlorophenol, *per se*, inhibits oxidative phosphorylation, and as a result fever, profuse sweating, an increased metabolic rate, and weight loss occur in cases of poisoning (Chapman and Robson, 1965; Bergner *et al.*, 1965). However, toxic effects such as chloracne associated with neurologic pain of the lower extremities and bronchitis in humans (Baader and Bauer, 1951) may be the result of exposure to toxic trace contaminants. It is also possible that the hepatocellular necrosis observed in fatal human cases (Truhaut *et al.*, 1952; Gordon, 1956) is caused by toxic trace contaminants. Vacuolation of hepatocytes was noted in livers of infants that were exposed to technical grade pentachlorophenol in a nursery (Robson *et al.*, 1969). Vacuolation of rat liver cells was subsequently reported in experimental studies with a pentachlorophenol contaminated with 200 ppm of octachlorodibenzo-*p*-dioxin and "pre-octachlorodibenzo-*p*-dioxin"; but it was not stated in the paper whether other contaminants were also looked for (Knudsen *et al.*, 1974). It is difficult to determine in retrospect which of the toxic effects reported in the literature are truly caused by pentachlorophenol and which are due to toxic contaminants. Our results suggest that the contaminants cause most of the alterations reported in rat livers.

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